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Microsporogenesis in Datura Stramonium

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(WITH PLATES 8 AND 9)

It has long been known that the representatives of the genus *Datura* furnish interesting material easily available for plant experimentation. In addition, it has been pointed out recently (Blakeslee & Avery 2, 3) that this genus also affords an excellent field for the study of Mendelian inheritance and mutative variation. The general morphology of the members of the genus is a point of common knowledge, but so far as the writer is aware, no careful cytological studies have been made upon the reduction divisions of any of its members. Guignard (7) in his now classical work treats of double fecundation in *D. laevis*. Bönicke (4) has examined the prophases and counted the chromosomes in *D. Stramonium*. Aside from these two investigations, the work that has been done upon *Datura* has been of a pharmaceutical or of an experimental character.

The abundance of specimens of *D. Stramonium* near at hand during the summer of 1915 led the author to prepare a large number of anthers for a study of microsporogenesis in this plant. The material was killed, fixed, and embedded in paraffin, in which condition it was kept until the summer of 1919, when an increased interest in the plant as a teaching type induced the belief that a completion of the work and a publication of the results would be of interest. Accordingly, during the months of June and July,

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additional material was secured and the study of the problem renewed.

Material.—The 1915 collection was made from the purple-colored, spiny-fruited plants found growing in an abandoned barnyard near Delaware, Ohio; that collected in 1919 came from plants growing on the Indiana University campus and in a neighboring garden. The plants from both of the latter places were from seed furnished by A. F. Blakeslee. Those in the garden plot were known to be homozygous for the dominant characters, purple color and spiny fruit. The plants on the campus had come about through a series of rather indiscriminate crosses between the purple, spiny-fruited type and the green, smooth-fruited plants described by Blakeslee (2). In making collections from this plot care was taken to select flowers from plants showing the two dominant characters, but since the ancestry was not definitely known, some anthers used may have been taken from heterozygous plants. A careful consideration of the stained cells from the three sources failed to show any appreciable distinction and no attempt was made to keep the preparations separate, but most of the work was done with the Blakeslee strain.

Method.—Parts of the corollas bearing stamens with anthers in various stages of development were killed in the chromo-osmo-acetic and chromo-acetic solutions, washed, dehydrated, embedded in paraffin, and cut into sections 3–5 μ thick. The killing fluid containing osmium gave uniformly better fixation and, in the latter part of the work, its use was adhered to strictly. Haidenhain's haematoxylin and the modified triple stain were used. The orange G of the latter combination was used in clove oil as recommended by Chamberlain and others.

Adaptability of material.—The pollen mother cells of *Datura* offer fair conditions for cytological investigation. Since the anthers in a bud mature their pollen simultaneously, it is necessary to examine a large number to secure the various stages. Some difficulty is attached to the removal of the young cells for the preliminary examination. This probably explains why they have not been examined cytologically before. The sporogenous cells are rather small and have very dense cytoplasm, but the chromatin material is fairly abundant for a dicotyledonous plant. Com-

paratively few chromosomes are formed and the cells stain readily with the usual laboratory dyes.

Anthers.—When the sporogenous cells are developing, the anthers range from two to four millimeters in length; those six millimeters long almost invariably contain mature pollen. Each anther has four loculi, the walls of which are composed of four layers of flat cells. As the anthers mature the cell walls of the two middle layers of these cells become thickened in the fashion usual for mechanical tissue and finally aid in the liberation of the pollen grains. The fourth layer becomes modified to form the tapetum which immediately surrounds the sporogenous cells.

Tapetal cells.—The cells of the tapetum are about the same size as the pollen mother cells when the latter are in the resting condition. At this time they are uninucleate, but by the time the mother cells have reached the spirem stage, they usually contain two or more nuclei, which have arisen from the original one by fragmentation. The tapetal cells increase in size as the spore-producing tissue develops, sometimes attaining a diameter two or three times that of the sporogenous cells. During this development the cytoplasm of most of the cells becomes decidedly vacuolated and, as it continues, the majority of the cell walls break down, and the cell contents are allowed to escape into the pollen cavity. Some of the cells apparently become reduced in size without any evident breaking of the walls, and still others seemingly persist unchanged until the pollen is matured.

The periplasm was carefully searched for "wandering cells" such as were found by Duggar (6) in *Symplocarpus* and more recently in *Galium* by Juel (8), but no positive evidence of such was found. Occasional cells were observed which had apparently broken loose from the anther wall. These were always angular in outline and were attributed to faulty manipulation rather than to any evidence of vital phenomena. Tapetal nuclei are not abundant in the periplasm; sometimes they may be seen as elongate, irregularly shaped structures, similar to those described by Pickett (13) for *Arisaema*, but more frequently as mere darkly staining fragments. No amoeboid forms were found.

Sporogenous cells.—The pollen mother cells arise directly from the primary sporogenous cells without division (Coulter & Cham-

berlain, 5); this accounts for their comparatively small number. In the resting stage they are about 25 μ in diameter and have large prominent nuclei. The cytoplasm is very dense and in the stained preparations seems to be composed of minute particles closely placed. As development proceeds these particles become more or less clumped and arranged in strands as shown in the figures. No evidence of mitochondria or other extra-nuclear bodies was found, but it is not doubted that such might be revealed if proper fixing and staining methods were used.

Nucleus.—In the so-called resting stage the nucleus has a diameter about two thirds that of the containing cell. It usually has a single darkly staining nucleolus, but occasionally two are found. The linin reticulum is composed of very delicate fibers upon which the chromatin material is arranged in the form of minute bodies (FIG. 1). In the preparations the threads are sometimes broken or else do not take the stain properly, but there seems to be no regular arrangement of either the threads or the chromatin bodies upon them. The material collected was too far advanced to show the telophase of the preceding division, but to all appearances the chromosomes of this phase lose their individuality in the maze of fibers and very small and numerous granules seen in the resting stage. It would seem impossible to trace a definite set of these fibers and chromatin masses to a particular chromosome, as Nothnagel (11) was able to do in the case of *Allium*.

FROM RESTING STAGE TO SPIREM

The synaptic condition is reached while the anthers are still quite young (FIG. 4). The initiation of this stage is marked by a thickening and consequent contraction of the linin threads (FIG. 2), which when continued results in their withdrawal from the periphery of the nucleus as shown in FIG. 3. Before the thickening has proceeded far the threads stain readily and their ramifications may be made out easily. The stage represented by FIG. 2 was studied carefully for evidence of the two spirem threads reported by Bönicke (4) for this plant. It will be noticed that the threads apparently anastomose freely and occasionally approximate as shown in the middle of the figure. Whether or not the two ap-

proximating threads occasionally seen should be considered spirem threads in the sense that Bönicke uses the term the writer is unable to determine. This approximation cannot be observed in every cell in this stage of development; it is more in evidence in the figure than is ordinarily seen. In other parts of the same nucleus the threads seem to radiate from chromatin masses, and in still other places the threads seem to be formed by the union of three or more smaller ones. To the mind of the writer the effect is so confusing, that he feels that anyone with a bias in his thinking, no matter what it might be, could find an apparent basis for it here. FIG. 3 shows a stage a little later than that shown in FIG. 2. The linin-chromatin mass has withdrawn from the nuclear membrane and is enveloping the nucleolus. In case two nucleoli are present one of them may be left out of the synaptic ball. At this time the threads and chromatin masses have increased greatly in thickness and stain sharply. On account of the contracted condition the arrangement can not be made out so readily as in the stage just preceding.

Synapsis.—This term is used in botanical literature for the condition shown in FIG. 4. The nucleolus is closely enveloped in the chromatin mass, but its outline as well as that of the threads may be distinguished. The details of the threads, however, can not be made out even in the most carefully stained preparations. This stage occurs so regularly and the sequence is so evident that it is hard to believe that it is an artifact, as Schaffner (**14**) asserts for *Agave*. In it the formation of the spirem thread is consummated.

Hollow spirem.—FIG. 5 shows the spirem thread as it emerges from the synaptic ball. At this time it is rather thin and when stained with suitable density it appears granular in nature. If the stain is dense, it appears as a fairly smooth thread without any internal differentiation whatever. At this stage no evidence of the double nature of the thread could be made out. Later the thread becomes more loosely arranged in the nuclear cavity as shown in FIG. 6. So far as it has been possible to determine, it is endless.

FORMATION OF BIVALENTS; METAPHASE; ANAPHASE; TELOPHASE

The stage represented in FIG. 6 is followed by a contraction and consequent thickening of the strand. The slightly thickened

strand has a tendency to become arranged in loose loops, which are cut off to form bivalents. In this behavior *Datura* is strikingly similar to *Lilium* as described by Mottier (9). FIG. 7 represents an early segmentation stage. It may be observed from this figure that all of the bivalents are not formed by the cutting off of loops from the spirem thread but that some of them must arise from the straight loopless part of it. Shortly before the segmentation of the chromosomes is brought about, the double nature of the thread becomes evident for the first time (FIG. 11). When segmentation is complete the twelve bivalent chromosomes may be made out. These bivalents may assume a number of different shapes as shown in FIGS. 9 and 10. Some of them appear as closed rings, others as U's, and still others have the chromosomes twisted about each other. The method of forming these various types is easily seen. The loops cut off from the spirem (FIG. 7) form the rings by the union or overlapping of their ends. If the ends are not united or overlapped the U-shaped form results, while the twisted form of bivalent results from the contraction of a loop with a twist in it. Payne (12) has shown that in the European earwig ring-shaped chromosomes may arise in three different ways in the same individual or even in the same cell. So far as it has been possible to determine, the method given above is the only one followed in *Datura*. In *Agave* (14) Schaffner was able to find a constant number of the various types of chromosomes present. A careful consideration of the cells of *Datura* fails to show any regularity in this respect.

Preceding the metaphase the bivalents appear in the multipolar arrangement so often described. This stage is of very short duration and difficulty is experienced in securing it. In the second division it is much more evident (FIGS. 16, 17). In the metaphase the bivalents become arranged in the usual fashion in the middle of the cell. In a polar view they are seen to have a regular distribution (FIG. 13). In this stage the chromosomes appear more or less heart shaped when viewed from the side (FIG. 12) and closely resemble Mottier's figure for *Helleborus* (10). There is no separation of the two halves of the chromosomes on the way to the poles, although the longitudinal division has doubtless taken place (FIG. 11). FIG. 14 shows a telophase stage with the chromosomes

still undivided. The volume of the chromatin material is considerably reduced between the segmentation of the chromosomes and their separation in the anaphase (FIGS. 6, 7, 8, 9, 11). As usual in the dicotyledonous plants a cell wall is not laid down till after the second division (FIG. 18).

SECOND DIVISION; MICROSPORES; POLLEN GRAINS

The material from which this study was made was taken from plants that were growing rapidly, and in it the chromosomes do not become diffused nor in any way lose their individuality between the first and second divisions. It sometimes happens that they become arranged to form a sort of a broken spirem, but each individual may be made out in it easily. This does not seem to occur with any regularity and the relation established is an approximation rather than a fusion of material. Whether or not the chromosomes behave any differently when growth is less rapid has not been determined. FIG. 15 shows a stage slightly further advanced than that represented by FIG. 14. Here the double nature of the chromosomes due to longitudinal splitting or separation is very evident. The next step in the process is a breaking down of the nuclear membrane and the formation of a typical multipolar spindle. When this multipolar condition first appears the chromosomes are seen to be more or less clumped in the midst of the weft of fibers. At this time (FIG. 16) the double nature of the chromosomes is somewhat obscured, but soon they become separated somewhat and their true nature may be made out easily (FIG. 17). In the bipolar spindle which soon follows, the chromosomes are arranged in a very exact fashion, giving the cell an almost diagrammatic appearance. In the following anaphase each chromosome is parted longitudinally and the halves started towards opposite poles. Following the telophase of this division (FIG. 18) a nuclear membrane is formed and the substance of the chromosomes again becomes arranged in the form of a reticulum similar to that seen in the resting condition of the mother cell. The material at hand does not show the details of this process. The walls separating the four cells appear about the time that the nuclear reticulum is established. The cells of the tetrads thus formed cling together for some time before separating as microspores. Shortly after

their separation the nucleus of each divides to form the generative and tube nuclei. Meanwhile the cell has increased in size greatly and a large central vacuole has been formed. A plasma membrane is formed about a portion of the cytoplasm containing the generative nucleus, thus forming the generative cell and completing the development of the pollen grain.

DISCUSSION

It is felt that anyone giving a cytological paper on a field so well-worn as that of the reduction divisions should have a distinct purpose in so doing. It is evident that such work can not reveal much that is strikingly new. A consideration of the literature of plant cytology shows that while many plants have been investigated few of the results have been correlated with the results of experimental work. Many of the plants studied cytologically have been monocotyledonous and not readily amenable to Mendelian investigation. Blakeslee (2) has shown that *Datura* has at least two pairs of characters that show Mendelian behavior with almost mathematical exactness. Such a result must be due to a precise handling of the hereditary substance and to the selection of unmistakeable characters by the plant breeder. The present work shows that there is a very definite apportionment of the chromatin material in the reduction divisions of *Datura*. After the chromosomes are cut off from the the spirem thread they may be traced as distinct individuals till the telophase of the second division. It is evident that, if the factors representing the parental characters are segregated in the two halves of the spirem thread, they would become equally distributed in the formation of the four microspores.

It has long been known that in the insects certain definite characters like sex may be traced to a specific chromosome or part of a chromosome in the reproductive cells. Recently Allen (1) has reported that a similar basis for sex may be made out in *Sphaerocarpos*, one of the liverworts. To one who has studied the cells of *Datura* it is clear that there is no visible evidence of such a basis for the Mendelian characters with which Blakeslee and Avery worked. The determinants of these characters must be chromosomes or parts of chromosomes that are for the present

indistinguishable. In this study no irregularity of chromatin behavior has been found that could explain the origin of mutants, but this could scarcely be expected since their occurrence is comparatively rare. It is conceivable that such might result from the distribution of the chromatin substance in megasporogenesis. If such is the case, it must for the present remain a closed book on account of technical difficulties and the laboriousness of the investigation.

The most striking thing about the chromosomes of *Datura* is their uniformity of size. This is especially evident in the second division. In the first division this uniformity is somewhat obscured by the shapes assumed by the bivalents until they appear in the metaphase.

Interpretation of the prophase.—The behavior of the chromatin material in the formation of the spirem thread has been a much mooted question and it seems that the present study of *Datura* can not shed much light upon it. In the resting condition the nucleus presents such a maze of threads and granules that almost any interpretation might be given. Bönicke (4) claims that the spirem thread is formed by the union of two smaller threads. The writer came to the present problem with a similar theory. As shown elsewhere the confirmatory evidence is not wholly convincing.

No satisfactory theory has been formulated as to how the chromosomes control growth or impart the parental characters to the offspring. It was purposed to make a comparative study of the chromosomes of plants showing the dominant characters and of the chromosomes from plants displaying the recessive characters. Since the rate of growth in the two types is quite different it was conceived that there might be a physical, determinable basis for it in the chromatin substance of the cells. Through an unfortunate misunderstanding the plants showing the recessive characters were destroyed before material was collected for study. Seeds have been planted, however, and it is hoped that work on this phase of the problem may be begun soon.

SUMMARY

1. As a dicotyledonous plant *Datura Stramonium* is favorable for cytological investigation as well as for studies of Mendelian behavior.

2. Part of the bivalent chromosomes are cut from the spirem thread as loops, which if twisted, result when shortened in the twisted form shown in the figures; if the ends overlap, or unite, the ring-shaped form is developed; the U-shaped type may arise from a loop or by the subsequent bending of the straight part of the thread.

3. The report of twelve bivalents made by Bönicke is confirmed.

4. There is no loss of the individuality of the chromosomes from the time that they are cut from the spirem thread till the telophase of the second division. This is an unusually striking phenomenon in *Datura*.

5. The exact results obtained by plant breeders from this plant are attributed to the unusual regularity in the formation and behavior of the chromosomes.

6. No physical basis for the Mendelian characters considered can be found in the chromosomes; nor is any cause found for the occurrence of mutants.

In conclusion, the writer wishes to take the opportunity of expressing his very great obligation to Professor D. M. Mottier, of Indiana University, under whose direction this work was brought to completion, for encouragement and valuable aid given; and to the board of trustees of the Ohio Wesleyan University for a relief from teaching duties that has made possible the pursuance of this investigation.

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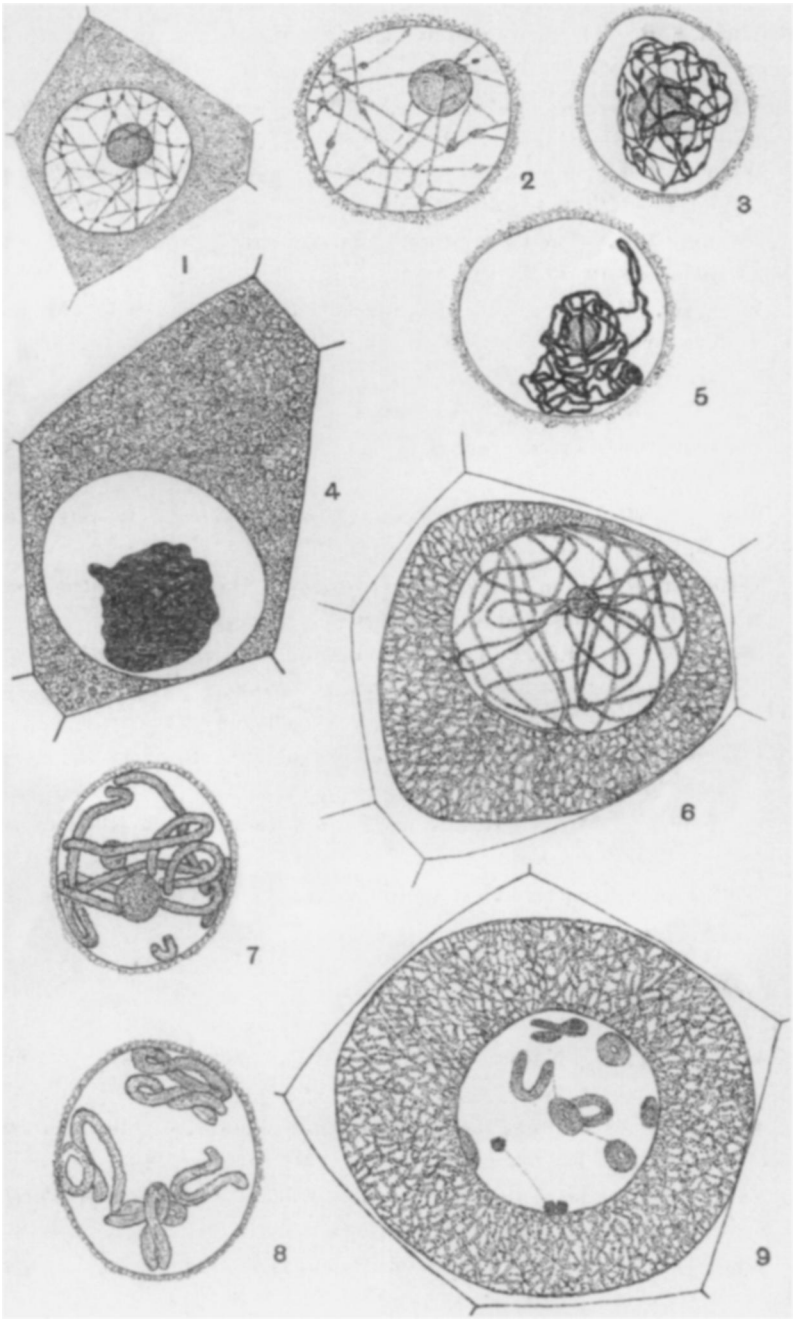
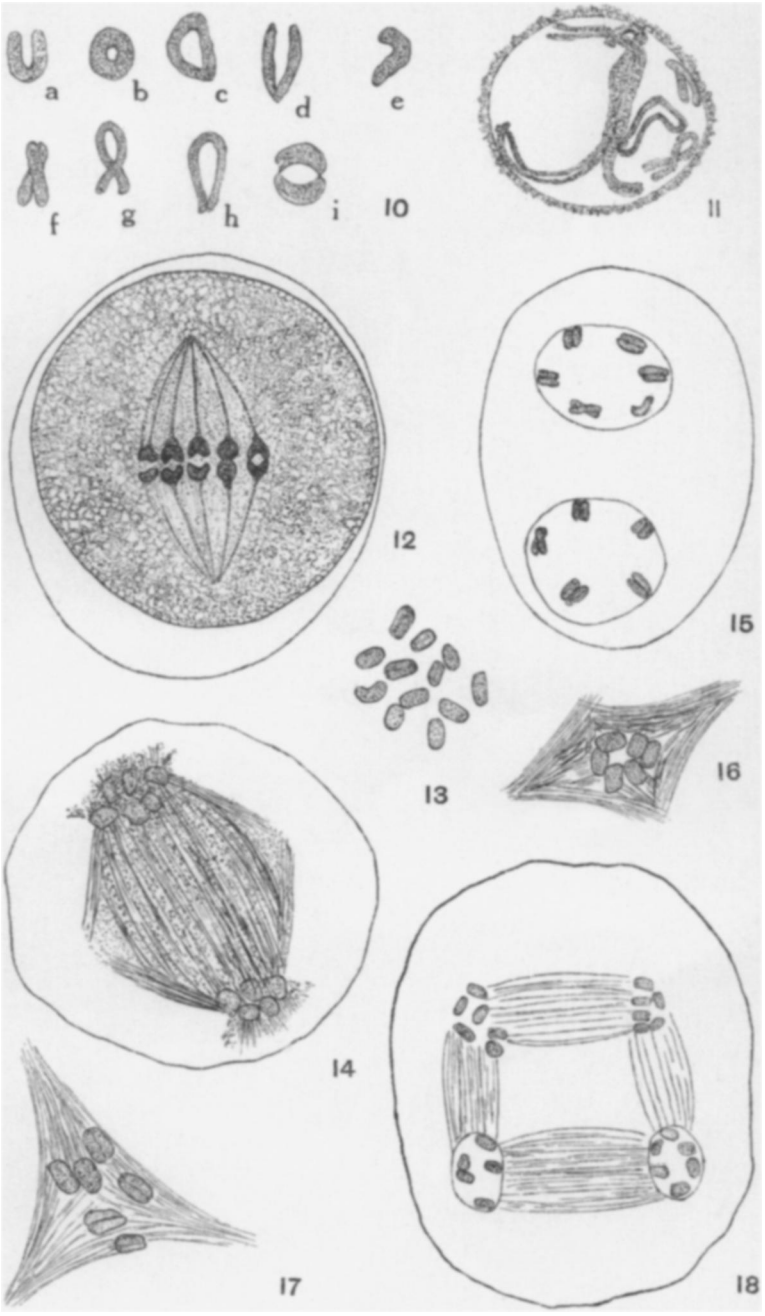


O NEAL: MICROSPOROGENENSIS IN DATURA



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Explanation of plates 8 and 9

All figures × 2270.

FIG. 1. A pollen-mother cell in resting condition.

FIG. 2. An early presynapsis stage.

FIG. 3. A stage a little later than that shown in FIG. 2.

FIG. 4. Synapsis.

FIG. 5. Coming out of synapsis.

FIG. 6. An early hollow spirem stage.

FIG. 7. Segmentation in process showing method of cutting off the bivalents.

FIG. 8. A stage a little later than that shown in FIG. 7.

FIG. 9. Segmentation.

FIG. 10. Some of the shapes assumed by the bivalents.

FIG. 11. A spirem stage in which the double nature of the thread is shown.

FIG. 12. A bipolar spindle, passing into anaphase.

FIG. 13. A metaphase, polar view, showing the twelve bivalents.

FIG. 14. A telophase.

FIG. 15. A little later stage, in which the double nature of the chromosomes is shown.

FIG. 16. An early multipolar spindle of the second division, showing the clumping of the chromosomes.

FIG. 17. A stage a little later than that shown in FIG. 16.

FIG. 18. Telophases of the second division.